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Analyzing Carbaryl Residue in Ponderosa Pine Bark by HPLC

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Carbaryl residue in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) was analyzed by high performance liquid chromatography (HPLC), with a UV detector absorbing at 280 nm. Although a simple Florisil column cleanup was adequate for high residue levels (> 50 ppm), interference problems occurred at lower levels. By introducing additional cleanup steps and converting carbaryl to 1-naphthol, analysis to 1 ppm was possible. These laboratory analyses are useful in assessing initial deposit and residual life of carbaryl when applied for control of selected bark beetle species.

Retrieval Terms: carbaryl residue, chromatographic analysis, bark, *Pinus ponderosa*, insecticides, carbamates

Carbaryl, a common agricultural carbamate insecticide, is also used to control bark beetles and other forest pests. The compound has been analyzed by colorimetry,[1,2,3] spectrophotofluorimetry,[4] direct gas chromatography (GC),[5,6] GC after derivatization,[7,8] and high performance liquid chromatography (HPLC).[9,10,11] Although direct GC works well for many pesticides, the technique is less satisfactory for carbaryl analysis because the compound is unstable at elevated temperatures. The problem of thermal degradation is avoided by the use of HPLC.

This note describes three methods for analyzing carbaryl in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) bark. At high levels (>50 ppm), the analysis was accomplished easily. At lower levels, however, stubborn interference problems occurred. Bark and other plant tissues from coniferous trees contain a variety of materials that are readily extracted with organic solvents. Because of their relative abundance (about 6 percent in bark) and wide range of polarities, the extracted materials can present a difficult problem in residue analysis. By introducing additional cleanup steps and converting carbaryl to 1-naphthol, analysis to 1 ppm was possible.

MATERIALS AND METHODS

Method 1. (for residues ≥ 50 ppm carbaryl)

Ponderosa pine bark (a composite from about 20 trees) was coarsely ground in a Hobart[12] food chopper in the presence of dry ice and stored at 3°C. A 5-g subsample of ground bark was extracted by homogenization in a Virtis blender, model 23, at high speed for 4 min with 60-ml $CHCl_3$:hexane (2:1). After filtration through Na_2SO_4, 42 ml was collected and passed through a chromatographic column (10.5 mm i.d.) packed with 10-cm Florisil.[13] Additional 35-ml $CHCl_3$:hexane (2:1) was passed through the column. All the eluate was collected and evaporated to about 1 ml in a 250-ml round bottom flask, with a rotary flash evaporator at 40°C. The evaporation was completed with a gentle stream of air, and 3.5-ml CH_3CN:H_2O (1:1) were added to the flask. After capping the flask with aluminum foil, the residue was dissolved with gentle heating. The flask was placed at 3°C for a minimum of one-half hour and the precipitated waxes and resins were removed by centrifugation and filtration through glasswool. After appropriate dilution the sample was analyzed by HPLC.

The liquid chromatograph (Waters Assoc.) used was equipped with a model 6000A pump, a 61-cm by 2-mm i.d. column, packed with Bondapak C_{18} Corasil, and a model 440 UV fixed wavelength detector absorbing at 280 nm. Conditions were:

mobile phase: $CH_3CN:H_20$ (40:60)
flow rate: 0.6 to 0.7 ml/min
sensitivity: 0.01 or 0.02 AUFS
recorder: Varian,model A25 at 1 mv.

Under these conditions the retention time of carbaryl was 3.5 to 4.5 min with a response of about one-third full-scale deflection to 10 ng carbaryl. Determinations were made on the basis of peak height measurements.

Method 2. (for residues of ≥5ppm carbaryl)

The extraction and cleanup steps by Florisil column were as described in method 1. After evaporating the eluate from the Florisil column, the residue was transferred to a 60-ml separatory funnel with two portions of 2-ml hexane followed by 2-ml 0.5 N NaOH, and 2-ml water. After shaking intermittently for 10 min, 5-ml $CHCl_3$ were added and, after shaking and phase separation, the organic solvent was drained and discarded. The Na-naphthoxide was converted to 1-naphthol by acidification with 0.5 N H_3PO_4 to pH 4 (pH paper). The 1-naphthol was extracted by shaking with 5- and 1-ml portions of $CHCl_3$. The combined $CHCl_3$ extracts were collected in a 100-ml round bottom flask and, after addition of 1-ml mixture of diethylene glycol:$CHCl_3$ (1:150), the sample was evaporated to about 1 ml. Evaporation was completed with a gentle stream of air. The relatively high volatility of 1-naphthol necessitates careful evaporations. Losses of 40 percent were found without the addition of diethylene glycol. After addition of 3.5-ml $CH_3CN:H_2O$ (1:1), the procedure for removal of waxes and resins and subsequent analysis by HPLC was as described in method 1. Retention time of 1-naphthol was 4 to 5 min with about one-third full-scale deflection to 10 ng 1-naphthol.

Method 3. (for residues of ≥ 1 ppm carbaryl)

Extraction and cleanup by Florisil column was as described in method 1. After evaporating the eluate of the Florisil column, the residue was transferred to a second 10.5-mm i.d. column, packed with Florisil, partially deactivated with water.[14] The residue was transferred with 5-, 5-, 10-, and 20-ml portions solvent A. (This solvent was prepared by shaking $CHCl_3$, hexane, and water[15] in a proportion of 65:35:50 and removal of the water phase.) The eluate was discarded. One hundred ml solvent A were added to the column and the eluate evaporated to dryness. The residue was transferred to a 60-ml separatory funnel for hydrolysis and liquid-liquid partitioning as described in method 2.

Recoveries and Controls

Five g untreated, coarsely ground bark were fortified with a known amount of carbaryl dissolved in 60-ml $CHCl_3$:hexane (2:1). The fortified sample was processed as described for each method. Samples were fortified at 1-, 5-, 50-, and 500-ppm levels (*table 1*). The recoveries were determined by comparing the processed samples with carbaryl or 1-naphthol standards at the expected concentration. Corresponding control samples were prepared from untreated bark, according to each method, to determine background response. Controls and fortifications at each level were replicated three times. Each sample replicate was injected three times to determine an average peak height.

RESULTS AND DISCUSSION

The percent recoveries ranged from 69.0 to 103.9 (*table 1*). The replicate figures for percent recovery at the 500-ppm level were 95.9, 123.4, and 92.3, with the 123.4 value contributing heavily to the high (17.0) standard deviation. The reason for the high reading could not be identified. The necessity for a larger number of replicates, therefore, should be considered.

A typical HPLC chromatogram of a standard, a fortified, and a control sample are provided (*fig. 1*). Control samples showed no peaks at the retention time of carbaryl or 1-naphthol. At levels corresponding to 5 ppm or lower, however, some bark extractives produced other peaks that prevented the use of an electronic integrator. Because no difference was found between standard curves (2-, 5-, 10-, and 20-ng 1-naphthol) determined by integration or peak height measurement, all determinations were made on the basis of peak height.

The methods described do not distinguish between residual carbaryl and 1-naphthol, a possible degradation product. However, 1-naphthol has not been found to be a major constituent of carbaryl residue in plants and its separate determination is not critical.[16]

Table 1—*Recoveries from ponderosa pine bark samples fortified with carbaryl at various levels and analyzed by three methods*[1]

Method	Carbaryl added (ppm)	Final volume (ml)	Recovery	
			Expected (ng)	Observed[2] (pct.)
1	500	350	[3]10	103.9 ± 17.0
1	50	35	[3]10	99.7 ± 4.9
2	50	35	[4]7.16	82.0 ± 2.6
2	5	3.5	[4]7.16	78.7 ± 2.5
3	5	3.5	[4]7.16	88.0 ± 0.4
3	1	3.5	[4]3.58	69.0 ± .4

[1]Injected volume of carbaryl was 2 μl for all trials except for that of method 3 with 1 ppm addition of carbaryl in which it was 5 μl.

[2]Data represents the average of three replications ± standard deviation.

[3]Carbaryl.

[4]1-naphthol (molecular weight ratio of 1-naphthol to carbaryl = 0.716).

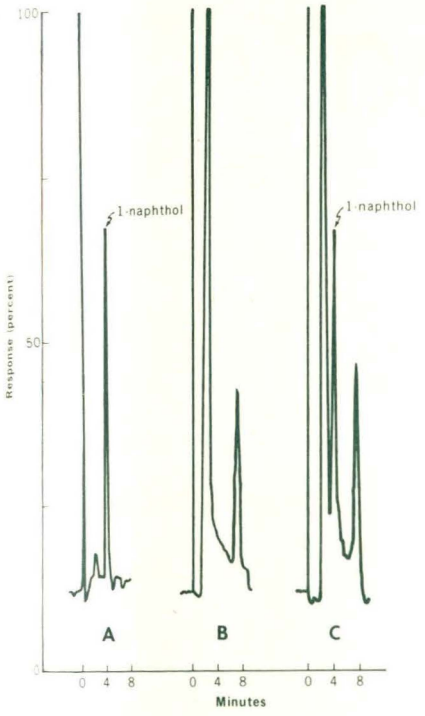

Figure 1—HPLC chromatograms of *(A)* standard, 7.16 ng 1-naphthol, *(B)* control, ponderosa pine bark processed by method 2 and *(C)* ponderosa pine bark fortified with 5 ppm carbaryl and processed by method 2. Control sample *(B)* showed no peak at the retention time of carbaryl or 1-naphthol.

The methods were developed to study the residual life of carbaryl in ponderosa pine bark. The insecticide was applied to tree trunks as a 1 percent a.i. aqueous spray for bark beetle control. Residues in bark samples taken up to 4 months after application, were high enough to allow the use of the simple cleanup described in method 1.[17]

NOTES

[1]Anonymous: 1974. Report: *A method for the determination of residues of carbaryl on plant foliage*. Union Carbide Corp. Agric. Prod. Dep., S. Charleston, W. Va. 4 p.

[2]Johnson, D. P. 1964. *Determination of Sevin insecticide residues in fruits and vegetables*. J. Assoc. Off. Anal. Chem. 47(2):283-286.

[3]Stansbury, H.A., and R. Miskus. 1964. *Sevin. In* Analytical Methods for pesticides, plant growth regulators, and food additives. Vol. II. Insecticides, p. 437-450. G. Zweig, ed. Academic Press, New York and London.

[4]Argauer, R.J., and R.E. Webb. 1972. *Rapid fluorometric evaluation of the deposition and persistence of carbaryl in the presence of an adjuvant on bean and tomato leaves*. J. Agric. Food Chem. 20(3):732-734.

[5]Hall, R.C., and D.E. Harris. 1979. *Direct gas chromatographic determination of carbamate pesticides using carbowax 20M-modified supports and the electroconductivity detector*. J. Chromatogr. 169:245-259.

[6]Riva, M., and A. Carisano. 1969. *Direct gas chromatographic determination of carbaryl*. J. Chromatogr. 42:464-469.

[7]Butler, L.I., and L.M. McDonough. 1970. *Specific GLC method for determining residues of carbaryl by electron capture detection after derivative formation*. J. Assoc. Off. Anal. Chem. 53(3):495-498.

[8]Khalifa, S., and R.O. Mumma. 1972. *Gas chromatographic separation of the aglycone metabolites of carbaryl*. J. Agric. Food Chem. 20(3):632-634.

[9]Colvin, B.M., B.S. Engdahl, and A.R. Hanks. 1974. *Determination of carbaryl in pesticide formulations and fertilizers by high-pressure liquid chromatography*. J. Assoc. Off. Anal. Chem. 57(3):648-652.

[10]Moye, H.A. 1975. *High speed liquid chromatography of pesticides*. J. Chromatogr. Sci. 13:268-279.

[11]Pieper, G.R. 1979. *Residue analysis of carbaryl on forest foliage and in stream water using HPLC*. Bull. Environ. Contam. Toxicol. 22:167-171.

[12]Trade names and commercial enterprises or products are mentioned solely for information. No endorsement by the U.S. Department of Agriculture is implied.

[13]Decomposition of carbamates on activated materials such as Florisil, alumina, or silicagel has been reported by Hall and Harris.[5] No decomposition of carbaryl on Florisil was observed in this study.

[14]Batches of Florisil may differ and the amount of water required for deactivation may vary from 0 to 10 percent.

[15]The water used in preparation of Solvent A removes ethanol, usually added to $CHCl_3$ as a stabilizer. Ethanol, even in small quantities, can appreciably accelerate the passage of carbaryl through the Florisil column. This can result in a poorly controlled elution pattern of carbaryl.

[16]Personal communication from R.R. Romine, Union Carbide Corp., S. Charleston, W. Va. 1979.

[17]Page, Marion, and G. René Pieper. Degradation and persistence of insecticides in ponderosa pine bark: II. Carbaryl, chlorpyrifos, fenitrothion and phosmet. (Manuscript in preparation.)

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